imprinting of *Igf2r*. On the paternal chromosome, an antisense RNA, *Air*, is transcribed through *Igf2r* from the opposite strand, leading to its silencing and methylation. Further studies have shown that the imprinting of two genes, *Slc22a2* and *Slc22a3*, that lie some distance upstream from the *Air* promoter also depends on the expression of *Air* RNA[8]. This shows that imprinted repression mediated by antisense RNA does not require transcriptional overlap. In a study where *Air* was truncated, paternal-specific silencing of *Igf2r*, *Slc22a2* and *Slc22a3* was completely lost, indicating that the *Air* RNA itself is important for silencing[9]. A similar control mechanism may be involved in the repression of *Xist* on the active X chromosome by the antisense transcript *Tsix*[10]. The *Xist* RNA has a key role in X inactivation. It becomes physically associated with the future inactive X over a substantial portion of its length, leading to long-range DNA methylation and chromatin condensation. The transcription of *Tsix* from one of the X chromosomes is involved in choosing the active chromosome by interfering with the accumulation in *cis* of *Xist*[10].

In both these examples of 'normal' antisense silencing, the repression only functions in *cis*, as in the case of the *HBA2* silencing[2]. A key difference in the case of the mutation associated with thalassemia is that the antisense RNA transcribed from the *LUC7L* promoter is spliced (including some alternative splicing) like the normal *LUC7L* mRNA, whereas the 108-kb *Air* RNA and 40-kb *Tsix* RNA are not and can interact collinearly with their transcription site. To determine whether the silencing and methylation are a general phenomenon or unique to the particular neighboring gene, Tufarelli *et al.*[2] replaced the *LUC7L* fragment with a human ubiquitin C promoter in one of their embryonic stem cell transfection experiments. In the antisense orientation, this produced the same silencing and methylation of the *HBA2* CpG island, suggesting the effect is general, although this result was seen in only 3 of 6 cases. This could be due to differences in promoter strength or an indication of some degree of RNA specificity.

A novel disease mechanism?

Recently it has become more widely recognized that the molecular mechanisms of genetic disease are varied. Genomic rearrangements outside the gene transcription domain have been frequently observed[11]. Most of these cases involve the interference with distant regulatory elements, whose function can be independently defined[12]. This novel α-globin rearrangement[2] adds a new mechanism to the list. It is intriguing to ask whether other examples of this mechanism are already out there, previously unexplained. The answer is probably yes: all that seems to be required is for a deletion or translocation to juxtapose a truncated, highly (ubiquitously?) expressed gene, lacking its poly(A)$^+$ signal, close to a disease-associated gene on the opposite strand. As ever, the difficulty lies in the detection of these cases. The disease gene and its normal regulation needs to be understood in fine detail—currently a rare situation.

1. Weatherall, D.J. *Nat. Rev. Genet.* **2**, 245–255 (2001).
2. Tufarelli, C. *et al. Nat. Genet.* **34**, 157–165 (2003).
3. Higgs, D.R. *et al. Semin. Hematol.* **35**, 93–104 (1998).
4. Barbour, V.M. *et al. Blood* **96**, 800–807 (2000).
5. Allshire, R. *Science* **297**, 1818–1819.
6. Mette, M.F. *et al. EMBO J.* **19**, 5194–5201.
7. Volpe, T.A. *et al. Science* **297**, 1833 (2002).
8. Zwart, R. *et al. Genes Dev.* **15**, 2361–2366 (2001).
9. Sleutels, F. *et al. Nature* **415**, 810–813 (2002).
10. Boumil, R.M. & Lee, J.T. *Hum. Mol. Genet.* **10**, 2225–2232 (2001).
11. Kleinjan, D.A. & Van Heyningen, V. *Hum. Mol. Genet.* **7**, 1611–1618 (1998).
12. Kleinjan, D.A. *et al. Hum. Mol. Genet.* **10**, 2049–2059 (2001).

Epigenetic interplay

Kye-Yoon Park & Karl Pfeifer

In a new study, the presence of a CpG island transgene protects neighboring sequence from DNA methylation during development leading to an open chromatin state that persists in the adult animal. This study suggests the importance of DNA methylation in setting up the epigenome.

The developmental program is controlled by genetic and epigenetic mechanisms. Epigenetic marks are those that are heritable through mitosis or meiosis but are not dependent on changes in DNA sequence. These marks can constitute molecular signals that distinguish between active and inactive genes. DNA methylation is one well characterized epigenetic mark, and mechanisms by which methylation can alter gene activity have been established[1,2]. Histone modification is a second potential mark. A great variety of histone modifications have now been identified, and their correlation with transcriptional states has led to the proposition of the 'histone code' hypothesis[3]. Histone modifications are conserved in all eukaryotes; however, their heritability is not well understood. In this issue, Hashimshony *et al.*[4] characterize an elegant genetic system to determine how epigenetic marks are established early in mammalian embryogenesis and control gene expression patterns throughout development. This study suggests that a crucial role for certain *cis*-acting regulatory sites is to prevent DNA methylation and induce histone modifications that permanently permit gene expression.

The authors are in the Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA. e-mail: kpfeifer@helix.nih.gov

Early epigenetic modifications

DNA methylation in mammals is a dynamic process[5]. Just after fertilization, the paternally inherited genome is actively and rapidly demethylated. Demethylation of the maternal genome lags behind and is probably passive and results from the lack of maintenance of methylation during the embryo's early cell divisions. Around the time of implantation, the somatic methylation pattern begins to be re-established by a wave of *de novo* methylation. After gastrulation, methylation patterns are relatively stable, although some changes are seen in cell culture, aging and cancer.

Most cytosines that are part of CpG dyads are methylated during embryogenesis[6]. The primary exception are cytosines that are part of extended clusters of CpG pairs known as CpG islands. There are 29,000 islands in the human genome associated with 5′ promoter regions of most constitutive genes and of

many tissue-specific genes as well. The exact mechanism by which these islands remain unmethylated is essentially unknown. Binding of Sp1 seems to be protective[7,8] and might occlude DNA methyltransferase directly or might act indirectly by inducing transcription or regional alterations in chromatin structures.

DNA methylation seems to affect gene expression through two mechanisms[1,2]. First, cytosine modification can directly interfere with DNA recognition by sequence-specific activators or repressors. Examples of this straightforward mechanism are actually rare[9,10]. More commonly, methylation seems to regulate gene activity by recruiting histone modifiers, and the altered chromatin structure directly represses transcription[1,2]. Analysis of the effect of cytosine methylation is complicated by the fact that nucleosome organization and histone modifications themselves can alter DNA methylation patterns, making it difficult to discern whether DNA methylation is the cause or the effect of transcriptional repression.

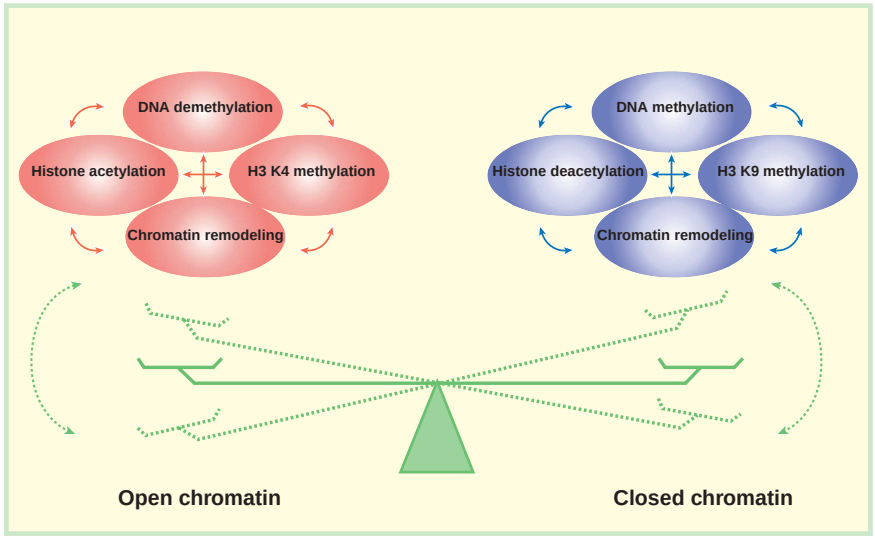

Figure 1 The epigenetic balance. Each epigenetic mark—DNA methylation, histone modification and nucleosome organization—can interact with and influence the establishment and maintenance of other marks. The chromatin state is determined by a balance between epigenetic and genetic programs favoring an open state and those favoring a closed state. Signals from the developmental program or the environment that change any one component may ultimately affect other components and shift the balance to a different chromatin state.

The role of CpG islands

Hashimshony *et al.*[4] (and see also ref. 11) used an *in vivo* genetic system to investigate the role of CpG island methylation established early in development in regulating gene expression. The authors compared RNA levels, DNA methylation and histone modifications of a human β-globin transgene in the presence and absence of a CpG island element flanked with *lox*P. Comparing transgenes in which the element was removed before implantation or not removed at all, Hashimshony *et al.*[4] found that the island element not only remained unmodified during the post-implantation wave of *de novo* methylation (as expected for a CpG island) but also protected the adjacent β-globin sequence. The protection from cytosine methylation was associated with high levels of transcription and histone modifications that are known to correlate with active transcription. In the next series of experiments, the authors removed the island element in adult tissues, after global methylation patterns have been established. In this case, the transgene remained active, undermethylated and with histone modifications consistent with active transcription. Thus, the island element represents a class of *cis*-acting factors that are activated at some particular point in development, initiating a cascade of events that organize the locus into a stably active or inactive region and then seem to become functionally unnecessary.

The authors interpret their results to indicate that the methylation pattern established in the embryo is key in setting up the structural profile of the genome. Strictly speaking, however, their experiments show that the island element, and not methylation, is associated with open chromatin structures. If the island element functions primarily to establish an open chromatin structure that is itself self-perpetuating, then one would predict identical results (assuming that DNA methylation can be a consequence of transcriptional repression). Both X-chromosome inactivation and repression of retroviral expression occur during early embryogenesis and are eventually associated with *de novo* methylation, which is essential to maintain gene repression. In each case, however, methylation occurs only after transcriptional repression[12,13]. Thus, methylation acts analogously to the Polycomb system in *Drosophila* in that it locks in expression patterns that are already established.

Cause and effect

The interrelatedness and the consequent difficulty in dissecting cause and effect relationships among DNA methylation, histone modification and nucleosome organization is a clear theme that emerges from numerous recent studies. DNA methylation can alter the histone code: for one example, DNA-binding proteins that specifically recognize methylated cytosine residues act to recruit histone deacetylase repressor complexes[14,15]. Conversely, DNA methylation levels are profoundly altered by mutations that disrupt histone modification or genes involved in histone–DNA interactions[1]. One way to consider these interactions is to emphasize the interdependence and assume that the feedback that one modification provides to another is crucial for establishing expression states at given loci (**Fig. 1**).

A novel approach for dissecting the exact effect of cytosine methylation at a given time and at a specific locus has recently been described[16]. In these experiments, introduction of a methylated oligonucleotide into established cell lines was able to induce methylation of the cognate (and adjacent) sequences in the chromosome. Methylation of the P4 promoter of human *IGF2* resulted in strong repression of *IGF2* transcription and even eliminated tumorogenicity of the cell lines. The mechanism for the ability of the modified oligonucleotide to induce methylation of the chromosome is not clear. The new methylation was not stable but lost after two generations in the absence of exposure to the oligonucleotide. This instability reflects the complex nature of the epigenome with its multiple reinforcing modifications that together maintain active and inactive chromatin complexes.

The study by Yao *et al.*[16] and that of Hashimshony *et al.*[4] attest to the functional interrelatedness of DNA methylation and

histone modifications—and our limited knowledge of how these mechanisms interact. Essential areas of future research include the characterization of mechanisms by which the cell modifies its epigenetic programs and the identification of mechanisms for therapeutic alterations of these programs.

1. Li, E. *Nat. Rev. Genet* **3**, 662–673 (2002).
2. Robertson, K. *Oncogene* **21**, 5361–5379 (2002).
3. Jenuwein, T. & Allis, C. *Science* **293**, 1074–1080 (2001).
4. Hashimshony, T., Zhang, J., Keshet, I., Bustin, M. & Cedar, H. *Nat. Genet.* **34**, 187–192 (2003).
5. Reik, W., Dean, W. & Walter, J. *Science* **293**, 1089–1093 (2001).
6. Bird, A. *Genes Dev.* **16**, 6–21 (2002).
7. Macleod, D., Charlton, J., Mullins, J. & Bird, A. *Genes Dev.* **8**, 2282–2292 (1994).
8. Brandeis, M. *et al. Nature* **371**, 435–438 (1994).
9. Ohlsson, R., Renkawitz, R. & Lobanenkov, V. *Trends Genet.* **17**, 520–527 (2001).
10. Takizawa, T. *et al. Dev. Cell* **1**, 749–758 (2001).
11. Siegfried, Z. *et al. Nat. Genet.* **22**, 203–206 (1999).
12. Wutz, A. & Jaenisch, R. *Mol. Cell* **5**, 695–705 (2000).
13. Pannell, D. *et al. EMBO J.* **19**, 5884–5894 (2000).
14. Jones, P. *et al. Nat. Genet.* **19**, 187–191 (1998).
15. Nan, X. *et al. Nature* **393**, 386–389 (1998).
16. Yao, X. *et al. J. Clin. Invest.* **111**, 265–273 (2003).

The advantages of recombination

Antonio Bernardo Carvalho

Many organisms have sexual recombination, yet its advantages are not fully understood. A new study of the fruit fly *Drosophila miranda* shows that recombination is essential for removing deleterious mutations from the genome and for allowing beneficial mutations to be incorporated.

Sexual recombination is the process whereby the genetic material of the parents is shuffled in meiosis and then mixed in fertilization. The question of its evolutionary advantage has been the subject of many theoretical and empirical investigations[1]. One powerful empirical approach has been the study of genomic regions that originally had a normal level of recombination and then lost it. The alterations induced by the loss of recombination may suggest its evolutionary advantages. The loss of recombination can be achieved experimentally with genetic tricks[1] or by relying on natural occurrences[2]. On page 215 of this issue, Doris Bachtrog outlines a study[3] that falls into the second category.

Recombination, here and gone

Around one million years ago, an autosome of the fruit fly *D. miranda* became attached to the Y chromosome, whereas its homolog retained its ability to segregate freely. *Drosophila* males lack recombination, and hence the attached autosome (called 'neo-Y') suddenly lost it. Owing to the mechanics of meiotic divisions, its homolog (aptly named 'neo-X') behaves as an X chromosome and is able to recombine in females, where, like the ordinary X, it is present in two copies. Thus, we have two large genomic regions that were identical a short time ago, and whose divergence is probably due to the effect of recombination. The beauty of this system has been noted[4], and work done by the Steinemanns group showed that the genes on the *D. miranda* neo-Y are degenerating[2]. The study by Bachtrog extends this work by fully applying population genetics theory and methods, and the overall breadth of the investigation has produced an elegant textbook example of the evolutionary advantages of recombination. The lesson is clear: "deleterious mutations…accumulate on a non-recombining chromosome, whereas positive selection (adaptive evolution) is confined to the recombining homolog"[3].

Sex and the neo-Y

Bachtrog[3] sequenced a region of approximately 11 kb (containing seven genes) of the *D. miranda* neo-X and the homologous region in the neo-Y. In accordance with previous studies[2], most genes on the neo-Y show various signs of degeneration, such as frameshift insertions and deletions, transposable element insertions and a high rate of amino-acid-changing mutations (amino-acid changes are usually deleterious to protein function). The neo-X versions of the genes are normal. This is in accordance with theoretical studies, which predict that natural selection is less efficient under low recombination[5], allowing deleterious alleles to persist and beneficial alleles to be lost. Bachtrog's study detected both effects. As already mentioned, the neo-Y accumulated many deleterious mutations. What about the beneficial alleles? The evidence here derives from observations of the neo-X and is indirect. Six of the neo-X genes showed no amino-acid substitutions since the neo-X/neo-Y split, which is expected for functional genes (given the short divergence time). Notably, the neo-X copy of the gene *exu-1* had a very high rate of amino-acid substitutions and other signs that suggest that natural selection is fixing beneficial alleles. A previous study[6] has detected an excess of amino-acid replacements on another neo-X gene (*CycB*). Hence, fixation of beneficial alleles ('positive selection') seems to be more frequent on the neo-X. Given the randomness of mutation, some beneficial alleles had probably arisen in the neo-Y but in the absence of recombination, natural selection

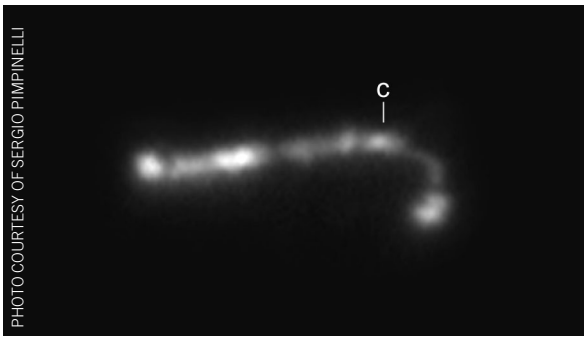

PHOTO COURTESY OF SERGIO PIMPINELLI

Figure 1 The Y chromosome of *Drosophila melanogaster*. Non-recombining chromosomes shed light on the advantages of sexual recombination. The chromosome in this image is stained with DAPI.

The author is at the Departamento de Genética Universidade Federal do Rio de Janeiro, Caixa Postal 68011, Rio de Janeiro, 21944-970, Brazil. e-mail: bernardo@biologia.ufrj.br